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Treatment of Classical Kaposi's Sarcoma With Intralesional Injections of Cidofovir: Report of a Case

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The effect of intralesional injections of cidofovir, a nucleotide analog with potent in vitro activity against human herpesvirus 8 (HHV-8), was studied in vivo in an HIV-negative patient with classical Kaposi's sarcoma (KS). After five weekly injections of the drug, no clinical, histological, immunohistological, or virological changes could be detected in comparison with saline-injected lesions. These findings suggest that, once the KS tumor has developed, active viral replication is no longer involved in the pathogenesis of the disease. Alternative hypotheses are that HHV-8 replication in blood-borne cells may foster growth of spindle cells in the skin, or that blocking HHV-8 may not affect existing lesions but may prevent new lesions from developing. ***J. Med. Virol. 55:215–218, 1998.***

KEY WORDS: bcl-2; human herpesvirus 8; nucleotide analog; p53 protein; viral replication

INTRODUCTION

Kaposi's sarcoma (KS) is an angioproliferative disease characterized by the presence of spindle-shaped cells that are considered to be the "tumor" cells of KS. The recent detection of a new herpesvirus, designated human herpesvirus 8 (HHV-8), in the various epidemiological forms of KS, strongly suggests that this agent may be involved in the pathogenesis of the disease [Chang et al., 1994; Noel et al., 1996]. Despite substantial advances in the understanding of the structure and function of the new virus, its exact role in the process of tumorigenesis remains to be determined [Bigoni et al., 1996; Viviano et al., 1997].

If HHV-8 was directly involved in the pathogenesis of KS, it would potentially represent an attractive therapeutic or preventive target. Current data addressing this point are not conclusive. Retrospective analyses of KS incidence among HIV-infected patients, together with anecdotal reports of remission of KS, suggest that foscarnet may be of value in preventing or treating KS [Morfeldt and Torssander, 1994; Jones et al., 1995; Mocroft et al., 1996]. At the same time, many other patients with KS have received foscarnet or other antiherpesvirus drugs without apparent resolution of their KS [Costagliola and Mary-Krause, 1995]. We investigated the in vivo effect of cidofovir (Vistide), a nucleotide analog with antiangiogenic activity [Liekens et al., submitted for publication] and a broad-spectrum antiviral activity against DNA viruses (herpesviridae, adenoviridae, poxviridae, hepadnaviridae, and papovaviridae) [Hitchock et al., 1996]. Potent in vitro activity of cidofovir against HHV-8 was recently demonstrated [Kedes and ganem, 1997; Medveczky et al., 1997; Neyts and De Clercq, 1997]. Cidofovir, when injected intralesionally, has been shown to be highly effective for the treatment of human papillomavirus-mediated tumors [Van Cutsem et al., 1995; Snoeck et al., in press]. Besides the absence of toxicity, the advantage of this route of administration is to achieve higher drug concentrations into the lesion than could be reached by systemic administration. Since HIV may play a role in the pathogenesis of KS [Ensoli et al., 1994], we wished to investigate the effect of the antiherpesvirus drug on KS in herpesvirus drug on KS in an HIV-negative patient to minimize confounding factors.

CASE REPORT

A 75-year-old heterosexual Sicilian man was referred with a one-year history of purplish papules and plaques involving both legs. He was otherwise suffering from a severe asthmatic bronchitis whose therapy

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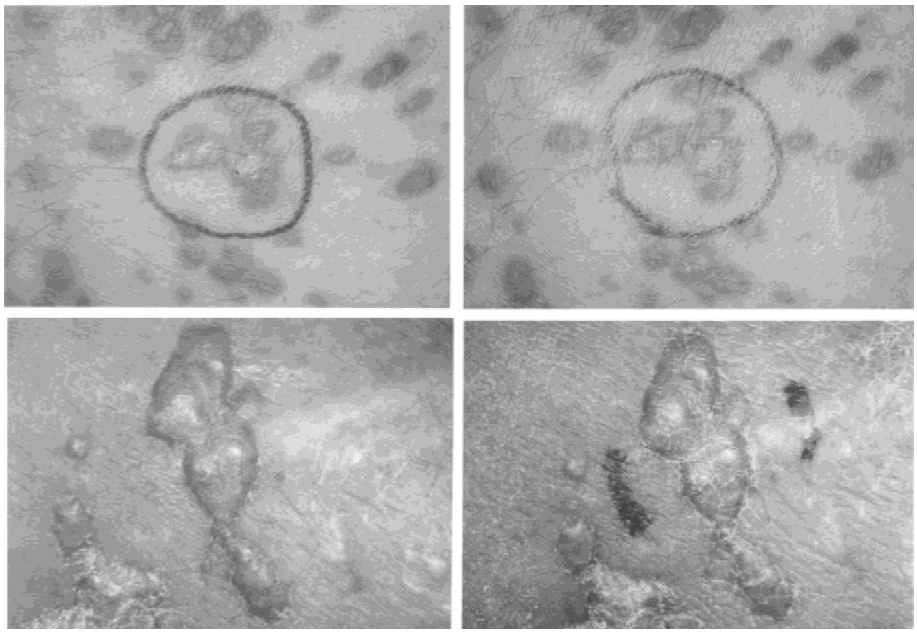

Fig. 1. Papular **(up)** and nodular **(down)** KS lesions before **(left)** and after **(right)** five weekly injections of cidofovir. No apparent morphological difference can be noted.

was dependent on corticosteroids (e.g., 5 to 40 mg of prednisone per day for 15 years). Skin biopsies of papules and plaques confirmed the diagnosis of KS. Serological testing for HIV-1 and -2 was negative. Routine hematological and biochemical investigations were normal. After informed consent, the patient was treated with intralesional injections of a 2.5-mg/ml solution of cidofovir. Using a bilaterally paired comparative approach, one macule (early-stage KS), one papule, and one nodule (late-stage KS) were injected with cidofovir on one leg and with normal saline on the other leg. Each lesion was injected with 0.8 ml once a week for 5 weeks. For intralesional administration, a 30-gauge needle was placed immediately under the skin overlying the KS lesion. The fluid was injected slowly to develop a wheal. The injected lesions were measured and photographed each week during the period of treatment. One week after the final injection, two cidofovir- and two saline-treated lesions (one macule and one nodule for each side) were biopsied. Each sample was divided into two parts, which were processed either for histopathologic examination or for culture. The saline- or cidofovir-treated papular lesions that were not biopsied were measured and photographed for an additional two-month period.

Frozen sections of both cidofovir- and saline-treated lesions were stained with hematoxylin-eosin for routine microscopic examination. The mean number of vessels per 10 to 15 fields was counted for each sample at ×400 magnification. The remaining fresh tissue was immediately stored at −70°C for subsequent immunohistochemical studies. p53 accumulation was detected with the monoclonal antibody DO7 (Dako, Glostrup, Denmark), using the antigen retrieval method as previously described [Kerschmann et al., 1994]. Bcl-2 expression was detected using the O-887 polyclonal antibody (Dako). Detection of proliferating cells was investigated using MIB1 (Immunotech, France), a murine monoclonal antibody against Ki67, using a method described previously by Cattoretti et al. [1992].

PCR was carried out as reported previously [Noel et al., 1996], using the KS330-233 primers described by Chang et al. [1994].

KS cell cultures were derived from two cidofovir- and two saline-treated lesions. Cultures were established similarly as described previously [Benelli et al., 1994; Simonart et al., 1996]. Briefly, cells were grown without additional growth factors in minimum essential D-Val (Gibco, Paisley, Scotland), 1% nonessential amino acids, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells isolated from these cultures were characterized as KS cells by morphological and immunohistochemical means (positive for laminin, vimentin, collagen IV, α-smooth muscle actin, ICAM-1; negative for keratin, ICAM-2, ELAM-1, VCAM-1, CD34, CD40, CD45) according to previously published reports [Benelli et al., 1994; Pammer et al., 1996; Simonart et al., 1996]. The experiments described now were carried out on cell cultures at the fourth passage.

RESULTS AND DISCUSSION

After five weekly injections of 0.8 cc of a 2.5-mg/ml solution of cidofovir, no apparent clinical improvement could be detected in comparison with the saline-injected lesions (Fig. 1). After an additional two-month follow-up period, no further changes could be demonstrated. No local or systemic side effects were noted. In particular, blood creatinine and urea values and measurements of creatinine clearance remained within

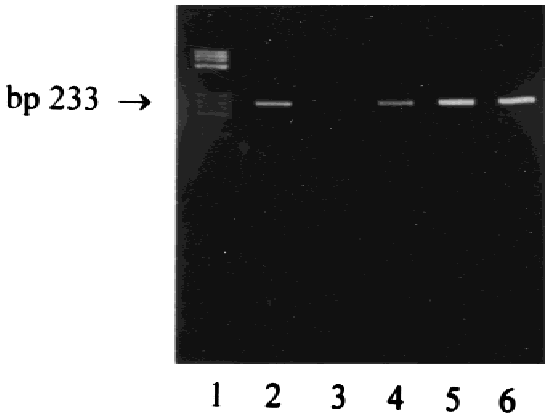

Fig. 2. Ethidium bromide-stained agarose gel of PCR products of KS samples. **Lane 1** shows the molecular DNA weight marker VI pBr 328-DNA. Early patch-stage KS lesion after five weekly injections of cidofovir **(lane 2).** Negative control (water) **(lane 3).** Early patch-stage lesion before the cidofovir therapy **(lane 4).** Late nodular-stage KS lesion before **(lane 5)** and after the cidofovir therapy **(lane 6).**

normal values during the whole therapy and follow-up period.

Routine histologic examination revealed no morphologic change in the cidofovir-treated lesions. There was no change in the number of blood vessels and vascular spaces. Neither necrosis nor aggravation of the inflammatory infiltrate were noted. The p53 and Bcl-2 proteins have recently been suggested to play a role in the pathogenesis of KS [Scinicariello et al., 1994; Bohan Morris et al., 1996; Noel et al., 1997]. Using immunohistochemistry, discrete p53 immunostaining of the nuclei of the spindle cells in nodular-stage (late KS) were found, but not in patch-stage (early KS) lesions. Bcl-2 staining revealed cytoplasmic reactivity within spindle-shaped cells. No significant differences were noted between cidofovir- and saline-treated lesions. To further assess the effect of cidofovir on KS cell proliferation, we investigated Ki67 expression. The Ki67 mitotic index did not vary between cidofovir- and saline-treated lesions.

Because cidofovir is able to suppress the in vitro replication of HHV-8, we next investigated whether HHV-8 could still be detected after therapy. Using polymerase chain reaction (PCR), we found that therapy with high, intralesional doses of cidofovir did not affect the presence of the viral DNA sequences. Semiquantitative analysis of the bands revealed no significant difference between the cidofovir- and the saline-treated lesions (Fig. 2).

In vitro analysis of spindle cells derived from cidofovir- or saline-treated KS lesions showed no difference in terms of cell proliferation (as determined by in vitro cell growth and by Ki67 staining).

Although HHV-8 DNA is present in all the progressive histological stages of KS [Chang et al., 1994; Noel et al., 1996], it is not clear whether the association between the virus and the tumor is causal or accidental. Since all antiherpesvirus drugs, including cidofovir, are only effective during the active viral replication phase, the findings support the view that HHV-8 persists in KS cells in a form that is not dependent on viral replicative enzymes (i.e., DNA polymerase). The lack of any apparent effect of cidofovir on early- and late-stage cutaneous KS tumors, as noted here in an HIV-negative patient, needs however to be further documented by additional cases; longer therapy and/or follow-up periods might be necessary to observe some effect. If confirmed, the data should be interpreted to mean that active replication of HHV-8 is not required for the maintenance of KS tumors, and, hence, cutaneous KS tumors, once established, may not be amenable to therapy with antiherpesvirus agents such as cidofovir. Further studies are required to determine whether parenteral cidofovir therapy could block the replication of HHV-8 in other sites (such as blood mononuclear cells), whether cidofovir may differentially affect KS lesions in HIV-positive as compared to HIV-negative individuals, and whether cidofovir may be effective in preventing the disease through interference with HHV-8 replication.

In conclusion, this study showed that under the conditions used, intralesional injections of cidofovir did not affect cutaneous KS lesions in an HIV-negative patient. Obviously, these data need to be confirmed by additional studies. Since cidofovir has a potent in vitro activity against HHV-8, our findings suggest that, once the KS tumor has developed, active viral replication is no longer involved in the pathogenesis of the disease. Alternative hypotheses are that HHV-8 replication in systemic (i.e., blood-borne) cells may foster growth of spindle cells in the skin, or that blocking HHV-8 may not affect existing lesions but may prevent new lesions from developing.

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